Amended Claims - Clean Copy

Patent Claims

1. Method for the two-dimension separation of biomolecules or other substance mixtures in gels, polymer carriers by means of electrophoresis, where
-the gels for the separation in the first dimension and the gels for the separation in the second dimension, arranged in succession or simultaneously vertical to one another, are brought into an electrophoresis combination chamber as casting gels or as ready-to-use gels and from each other are isolated by a hollow seal, polymerised and re-hydrated respectively,
-buffer solutions are then filled in, a biomolecule mixture is deposited onto the gels of the first dimension and the electrophoretic separation of the first dimension is carried out at constant temperature or at a fixed temperature gradient,
-the buffer solution is then suctioned off, the isolation neutralised, contact gel is filled into the resulting spaces between the first and second dimension and polymerised out, buffer solutions are filled in and the electrophoretic separation of the second dimension is carried out at a precisely set temperature and constant electric capacity or increasing current intensity, and
-finally, the gels are developed and the proteins are made visible by standard methods.

2. (amended) Method according to Claim 1,
wherein,
for the separation process, the gels in the electrophoresis combination chamber are standing vertically and the separation of the proteins is performed in the first dimension vertically and in the second dimension horizontally.

3. (amended) Method according to Claim 1,
wherein
the gels of the first dimension are cast as flat gels in a U-shaped tube, in which case a stop gel is first cast and, following this, the separation gel is cast and the casting processes as well as the polymerisation processes take place at constant temperature with activated cooling.

4. (amended) Method according to Claim 1

wherein

the gels of the second dimension are cast in two steps in such a way that, in a first step a sealing gel, and after its polymerisation, in a second step the gel solution is cast from below and rising in an upward direction in such a way that the air is displaced upwardly and the gel is finally polymerised at constant temperature with activated cooling.

5. (amended) Method according to Claim 1

wherein

the gels are produced with variable width and thickness.

6. (amended) Method according to Claim 1,

wherein

the neutralisation of the isolations is realised by physical removal of seals or switching by means of volume or diameter reductions of seal hoses.

7. (amended) Method according to Claim 1,

wherein

the process of the two-dimensional electrophoresis is performed automatically.

8. (amended) Method according to Claim 3,

wherein

the gels and the buffer solutions are tempered from the same cooling.

9. (amended) Device for the two-dimensional separation of biomolecules or other substance mixtures in gels by means of electrophoresis in an electrophoresis apparatus indicating electrodes,

wherein

an electrophoresis combination chamber (1) has a core (2) with cooling elements (3), where the cooling elements (3) are arranged between gel chambers (6, 7) and buffer vessels (8) formed on both sides of the core (2) by means of inner plates (4) and outer

plates (5) in combination with removable or switchable isolating elements in the form of a hollow seal (9).

10. (amended) Device according to Claim 9,
wherein
the cooling elements (3) are formed by means of a meander-shaped cooling labyrinth (10) with supply (11) and return (12) and the cooling labyrinth (19) encloses the buffer vessels (8) and (21), where the inner plates (4) are made of a good temperature-conducting material and the outer plates (5) are made of a transparent material.

11. (amended) Device according to Claim 9,
wherein
the inner plates (4) consist of ceramic or plastic material and the outer plates (5) of glass or transparent material, and the outer plates (5) are held in position by a clamping frame (13), and a cover (14) covers off the electrophoresis combination chamber (1) in the upward direction.

12. (amended) Device according to Claim 11,
wherein
the clamping frame (13) is secured on both sides by means of clamping elements (15) and has viewing windows (16) for process inspection.

13. (amended) Device according to Claim 9,
wherein
the lower limitation of the electrophoresis combination chamber (1) is realised by means of an adjustable and rotary table, on which the electrophoresis combination chamber (1) is fixed-positioned and the cover (14) indicates inlet and outlet lines for the cooling medium as well as to the buffer vessels (8, 21) and gel chambers (6, 7) and the connections for the electrodes of the first and second dimension.

14. (amended) Device according to Claim 9,
wherein

the core (2) consists of polymer material such as acryl glass, ceramic or plexi-glass, and the gel chambers (6,7) are joined with filling tubes (17), and vent openings are arranged, and between inner plates (4) and outer plates (5) seals are arranged, and the isolating elements (9) with recesses act together, and the parts of the electrophoresis combination chamber (1) contacting the media gels and/or gel solutions and/or buffer solutions are surface-coated, where the surface coating can consist of amorphous carbon layers.

15. (amended) Device for the two-dimensional separation of biomolecules or other substance mixtures in gels, polymers or carrier-free media by means of electrophoresis in an electrophoresis apparatus,

wherein

all assembly groups as required for the performance of a two-dimensional separation are fully integrated in an electrophoresis combination chamber (1), consisting of a core (2) with cooling elements (3), where the cooling elements (3) are arranged between the separating chambers (6,7) formed on both sides of the core by means of inner plates (4) and outer plates (5) in combination with removable or switchable isolating elements in the form of a hollow seal (9), buffer vessels (8, 21) and holders for the electrodes, and the performance of the two-dimension separation can be fully automated without the necessity of manipulation on the gels themselves during the course of the two-dimension separation.

16. Combination chamber for two-dimension separation of biomolecules or other substance mixtures in gels arranged horizontally and above each other by means of electrophoresis with a rear wall plate (28A) and a cover plate (28B), where at least two deflection elements (22) are arranged between rear wall plate (28A) and cover plate (28B) for guiding isolating elements in the form of a hollow seal (24).

17. (amended) Combination chamber according to Claim 16,

wherein

the chamber arrangement consists of an upper IEF-part for the performance of the IEF-electrophoresis in the first dimension and a lower part for the performance of the SDS-

electrophoresis in the second dimension, and the gels (25) and (36) are arranged horizontally above each other, and the plates (28A, 28B) are sealed off to the outside by means of seals (23), the configuration of the seals (23) and the thickness of the gels (25, 36) being fixed and, next to the deflection elements (22), electrodes (26, 27) are positioned for the electrophoresis of the first dimension, where the plate (28A) is made of ceramic or glass and the plate (28B) is a transparent plate.

18. (amended) Combination chamber according to Claim 16,

wherein

the rear wall plate (28A) forms a unit together with the upper buffer reservoir (29) of the second dimension as well as the pouring vessel (30) and the buffer filling vessel (31), and the assembled construction consisting of the plates (28A, 28B) and the upper buffer reservoir (29), the pouring vessel (30) and the buffer filling vessel (31) is placed and arranged in the lower buffer tank (32), and a seal (33) is arranged, which is liftable in function, and in the upper buffer reservoir (29) and in the lower buffer tank (32) electrodes (38, 39) are arranged for the electrophoresis of the second dimension.

19. Method for the two-dimension separation of biomolecules or other substance mixtures in gels or polymer carriers by means of electrophoresis in an electrophoresis combination chamber, where

- an IEF-gel is arranged horizontally in the combination chamber and is coated over with re-hydration buffer for re-hydration purposes,
- following this, a biomolecule or substance mixture specimen is brought into the IEF-gel or applied to the IEF-gel and the electrophoresis of the first dimension is performed,
- before, after or during the performance of the first dimension, an SDS-gel for the performance of the second dimension is brought in horizontally to the IEF-gel and, isolated from this by a hollow seal, into the combination chamber and the SDS-gel is polymerised,
- after completion of the IEF-electrophoresis the isolation is neutralised, contact gel put in to the resulting spaces, buffer solution added and the SDS-

electrophoresis in the second dimension is carried out, and finally the gels are developed and coloured according to the known methods.

20. (amended) Method according to Claim 19,

wherein

after the re-hydration of the IEF-gel, excess buffer solution is removed and the recess in the IEF-gel is produced by the introduction of a spacer during the re-hydration, and the IEF-gel is re-buffered after the electrophoresis by adding re-equilibration buffer, and the isolation is neutralised by the removal of a plastic hose by drawing out with the help of a stepping motor.

21. (amended) Method according to Claim 19,

wherein

the cooling of both electrophoresis-dimensions is performed by immersing the gel sandwiches in thermostatically controlled buffer solution of the second dimension, or the cooling of both electrophoresis-dimensions is realised by cooling chambers arranged in the combination chamber.

22. (amended) Method according to Claim 19,

wherein

the biomolecule or substance mixture specimen is brought into a recess in the IEF-gel.

23. (amended) Method for the one-dimension separation of biomolecules or other substance mixtures by means of electrophoresis,

wherein

- instead of the gel for the separation in the first dimension as well as the isolating element, a comb with specimen pockets for various specimens is placed in the SDS-gel and this is polymerised out,
- the comb is removed after out-polymerisation,
- the specimens are brought into the resulting recesses and
- following this, the one-dimension electrophoresis is performed.

24. Hydrated IEF-gel manufactured by pouring an immobiline gel with low pK on a gel-polymerised foil and its polymerisation, pouring of an acryl amide gel on the immobiline gel and incipient polymerisation, pouring of an immobiline gel with high pK on the acryl amide gel and its on-polymerisation and subsequent re-hydration by means of a re-hydration buffer, which contains 1-4% of such ampholines, allowing a pH-range within 2-11.

25. (amended) IEF-gel according to Claim 24,
wherein
the immobiline gels are manufactured from 6-10%, preferentially 10%, acrylamide with additive of 50-200 mM, preferentially 50-100 mM immobiline.

26. (amended) IEF-gel according to Claim 24,
wherein
the acrylamide gel is manufactured from 3,5-4,5% preferentially 3,5-5% acrylamide.

27. (amended) IEF-gel according to Claim 24
wherein
the re-hydration buffer contains of 5-9,5 M, preferentially 9 M, urea and, as required, detergents, preferentially Tween 20, Chaps or Triton X-100.

28. (amended) IEF-gel according to Claim 24,
wherein
before re-hydration, washing steps are carried out as required, and is dried.

29. Dry gel manufactured by pouring an immobiline gel with low pK on a gel polymerisation foil and its polymerisation, pouring of an acrylamide gel on the immobiline gel and its incipient polymerisation, pouring of an immobiline gel with high pK on the acrylamide gel and its incipient polymerisation.